An overview of microfluidic mixing application

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Abstract.

Microfluidics is a technology where application span the biomedical field and beyond. Single cell analysis, tissue engineering, capillary electrophoresis, cancer detection, and immunoassays are just some of the applications within the medical field where microfluidics have excelled. The development of microfluidic technology has lead to novel research into fuel cells, ink jet printing, microreactors and electronic component cooling areas as diverse as food, pharmaceutics, cosmetics, medicine and biotechnology have benefited from these developments. Since laminar flow is prevailing at most flow regimes in the micro-scale, thorough mixing is a challenge within microfluidics. Therefore, understanding the flow fields on the micro-scale is key to the development of methods for successfully microfluidic mixing applications.

Keywords: micro fluidic, mixing, application.

Introduction

Microfluidics can be defined as the study of fluids as they move through channels on a micro-scale. Behaviour of fluids at this scale differs from "normal" fluid behaviour. Things that have miniscule effects on larger scale systems start to dominate the system. Factors like surface tension, energy dissipation, and fluidic resistance strongly affect this system. Microfluidics, which can be studied using CFD (computational fluid dynamics) and experiments, is the study of how these behaviours relate and interchange in these systems, how they can be worked around, or exploited for entirely new uses.

This technology has been the topic for much research, as it provides a means for carrying out key chemical assessment processes in the biomedical field [1,2]. Due to the low volume of reagents and buffers needed for microfluidic analyses, and the speed of the analysis, these techniques have advantages over the standard bench-top methods [3,4,5]. In addition, by understanding the physics and phenomena of micro-scale flow, microfluidic devices can carry out experimentation and perform techniques that are not possible at the macro-scale, potentially allowing for new functionality and experimental systems to evolve [6]. Other advantages include the fact that microsystems are readily automated, parallelizable, portable, and have relatively low materials cost [7].

In most microfluidic devices, sample or reagents are moved through the enclosed microchannels in geometries that are embedded in chips. In addition to chemical assessment, microfluidics also give us an exciting set of tools for studying biology. There are many biological applications,

which have been miniaturised into a chip. These applications include DNA sequencing, DNA separation, polymerase chain reaction, electrophoresis, as well as cell sorting and cell counting.

Governing Equations

In fabricated microchannels the flow rate Q (m^3s^{-1}) is governed by the equation Q = ΔP/R Q=. Where ΔP is the pressure drop across the channel and R is the channel resistance. The R term takes into account the differences from channel to channel, such as surface resistance of the material or the channel geometries. It can be seen from this equation that there is a need for pressure difference to induce a flow rate. This can be created in various ways, but two methods commonly used in most applications are pressure driven flow and electro kinetic flow.

Pressure driven flow is flow created using an external pump or a vacuum source. This common method is not ideal for all applications like in assays which require high-resolution separation, because in this method a flow with a parabolic velocity profile is created which may not be ideal. Samples can then undergo axial dispersion and result in sensed peak broadening. The second method is electrokinetic flow. There are two parts to electrokinetic flow; electro phoresis which results from the accelerating force due to the charge of a molecule in an electric field balanced by the frictional force, and electro osmosis, which a uniform plug like flow of fluid in the channels. Equipment needed to induce electrokinetic flow is expensive but it also has many advantages. Unlike pressure driven flow, electrokinetic flow gives rise to flat velocity profiles which allows high resolution separations in capillary electrophoresis. In electrokinetic flow, the flow can also be controlled easily without the need for valves by simply turning on and off voltages. Its disadvantages include its incompatibility with some buffers, for this technique only certain strength pH and ionic strengths are compatible. There is also a need for a power supply off the microfluidic chip, and complications can arise from undesired heating of the fluid like evaporation of the solvent or formation of electrolytic bubbles. However, the high surface to volume ratio allows for large amounts of heat to be dissipated.

There are other methods for creating flow being explored. Chou et al. created a pneumatically actuated pump which mixed fluids from many inlets [8]. Ismagilov et al. designed a system pressurised by injection of a water-immiscible phase [9]. This generated uniform plugs, inside which the reactants mixed by chaotic advection.

Fluid Flow

Fluid flow occur in a laminar manner where the streams flow parallel and mixing only occurs by diffusion or by turbulent flow where stream lines cross and include fluid mixing. The Reynolds number is given by Re= vLρ/μ (where v is the mean fluid velocity in m x s^{-1}, L is the characteristic length in m, μ. is the (absolute) dynamic fluid viscosity in Nsm^{-2}, p is the density of the fluid kg x m^{-3}). This number characterises the presence of turbulent flow. If the number is extremely low, the flow will remain laminar. Therefore two fluids joining will not mix readily via turbulence, so diffusion must be relied upon to mix the fluid which is not ideal in most microfluidic applications [10]. To overcome this, static mixing geometry can be used to cause

increased mixing in the fluid. The introduction of sharp geometries can cause "eddy" currents to be generated which will cause fluid elements to get twisted and folded into one another; this folding increases the contact area between the two streams, increasing the rate of diffusion and mixing.

Applications of Microfluidics

Immunoassays

Immunoassays, most of which are heterogeneous, are a widespread way of detection of analytes using antibodies. When these immunoassays are prepared using microfluidic chips they minimize washing steps and fluid handling. An antigen-antibody is bound to a solid substrate and free antibodies are removed by washing. One disadvantage of heterogeneous immunoassays (i.e. an ELISA) is that they take a relative long time to perform. Sometimes they required hours for the laminar flow of analyte to diffuse from the solution to the surface.

By creating mixing geometries the diffusion distance can be minimized. Linder et al. detailed a process to create an immunoassay for detecting immunoglobulin (IgG) [11]. It was performed in a PDMS microchannel and required incubation times from 1 to 6 minutes. Typically these ELISA's assays for detecting antibodies are run manually many times for each antibody to be tested for. Therefore, in microfluids chips with many microwells, the sample can be run into various microwells within the same chip allowing for one serum to be tested for many antibodies.

Single Cell Analysis

Wheeler et al. focused on the use of microfluidics for single-cell analysis, they detail that controlled manipulation of cells is a problem that can be addressed with microfluidics [12]. Soft lithography as a manufacturing technique allows some advantages like, the addition of programmable valves, near zero dead volumes. Photolithographic techniques utilizing silicone masks and photoresist were used to create PDMS based micro-device. The device incorporated 5μm drain channels, 20μm valve seats patterned on top and 20μm cell delivery channels with digitally controlled manifolds. In the experiments flow rates of 300 to 1000m/s were used to analyse Jurkat and U937 cells. These cells are used to study acute T cell leukaemia. In these experiments a 488nm line argon ion laser was used. For the measurement of the flow rates and of the loading/perfuse switching times a small laser sport focused on the channel caused the dye in the solution to fluoresce. The cells were then excited by defocusing the laser to a 50μm spot by auxiliary optics. The fluorescence signal was filtered optically and spatially before being detected by a photomultiplier tube (PMT). The data was then transferred to a PC with LabView software.

This method allows rapid cell isolation from bulk cell population allowing precise delivery of reagents to desired cells. The cells were isolated at T-geometries using hydrodynamic focusing

using two buffer streams. The cells were docked in these T-junctions and here reagents were delivered and perfused easily. Only small amounts of expensive reagents were needed. In the process the reagent surrounds and encapsulates the cell. This device also allowed for the rapid, low volume implementation of traditional cellular analyses, such as receptor mediated cross linking dependant measurements on human myocytic U937 cells.

Cell sorting and cancer diagnosis

Microfluidic systems also have application in the sorting of cells and even in diagnosis of cancer. Studer et al. has performed in-depth research on the isolation of single cells [13]. They fabricated a device from PDMS using multilayered soft lithography. The device consisted of many active units (mixers pumps) pneumatically controlled by soft microvalves, see Figure 1. Using this fabrication a device can be created that can sort fluorescently tagged 10μm objects. Fluorescent events were first detected in a wide channel and then moved around in a sorting loop by a computer controlled pump. This pump can judge the volume of fluid displaced by one valve and as a result it can be very exact in its movements. Often in medicine rare cancerous cells can be tagged relatively easily. Using this method with several valves, one can create mixers, peristaltic pumps, switches, and multiplexers. This facilited system design makes locating or positioning the cell more easy. Many cells can be located by this method allowing for cell sorting or further biochemical assays such as cell lysis, mRNA extraction and polymerase chain reaction.

Capillary Electrophoresis

Microfluidics can also be applied to separating protein and DNA in techniques like capillary electrophoresis and liquid chromatography. There are advantages involved with this miniaturization like reduced time and cost. It also adds the possibility of using the components with other microfluidic components. For example, one might want to add a filter or components for extraction. Also as mentioned earlier electrokinetic flow has made capillary electrophoresis a popular technique as fluid flow can be controlled using voltages. Duffy et al. describes the technique for creating PDMS chips which use electrokinetic flow [14]. Once the geometry is created it is then plasma oxidized, this generates Silanol groups. Silanols are compounds containing silicon atoms, they are negatively charged at a neutral or basic PH. This allows electrosmotic flow towards the negatively charged cathode [15]. This electrokinetic flow acts as a sieving mechanism. This device can be used to separate amino acids and protein charge ladders. Although this method has proved successful in separation of DNA, other methods have also been used. One-dimensional sodium dodecyl sulphate (SDS) capillary gel electrophoresis (CGE) has been performed in a micro channel. Doyle et al.

compiled an in-depth review of the use of a stationary phase consisting of a self assembled magnetic matrix for separating DCA in a PDMS channel [15].

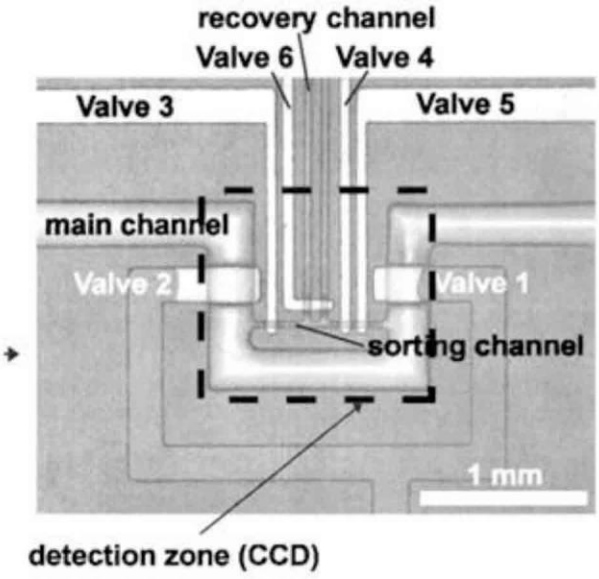

Figure 1: Single cell sorting geometry [13]

Combinational Screening

Sia and Witesides detailed a method that shows how microfluidics can be used for combinational screening [1]. A chip was prepared housing many microchannels; this chip was used to rapidly screen conditions for protein crystallization. Microfluidic chips are an ideal place for this screening as PDMS is gas permeable and allows complex channels to be filled with small amounts of protein sample with very few air bubbles. Compared to the conventional vapour diffusion method using a sparse matrix for sampling crystallization conditions, using a chip detected more conditions that generated crystals and used considerably less protein sample. This is a technology that is now successfully commercialized [1].

Effects of channel geometries in micromixer

Most microfluidic systems are too small to allow turbulence; thus, unlike ordinary size system, mixing can not rely on hydrodynamic fluctuations in microsystems. Mixing can not rely on molecular diffusion either, because microsystems are, in many cases, too large for diffusive mixing to be viewed as a successful or fast process. Modelling of turbulent microfluidic mixing is reviewed here and some methods suggested for creating turbulent flow in microchannels.

Chaotic micromixing

Dodge et al. proposed a method of using chaotic flow in a micromixer called the "cross channel micromixer" [16]. This paper is based on the idea that chaos is remarkably efficient at mixing low Reynolds number flows. In essence, the efficiency relies on the fact that, in chaotic systems, diffusive fluxes across interfaces are enhanced at exponential rates in time. In his investigation he

shows that the theory of chaos mixing is not a new idea and was developed to a high level in the 1980s [17]. It can be said however that microfluidics is a new application for these ideas. The device was composed of two main microchannels were two flows of glycerol were being mixed, one containing a fluorescent dye. These flows joined in laminar fashion but were perturbed by a transverse, oscillating periodic flow created at a cross-channel intersection, shown in Figure 2.

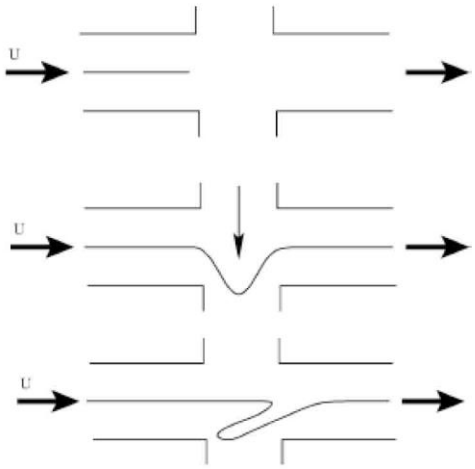

Figure 2: Effect of Oscillation at a T-Junction [16]

The oscillating flow was created using a pair of ten integrated PDMS valves. A water-filled actuation channel compressed a membrane which openedorclosed a fluidic channel. This deformation caused a liquid displacement directly related to the amplitude of the applied air pressure and the valve surface area. By switching on and off both valves intermittently, an oscillating perturbation at the cross-channel intersection was created.

Effect of geometry on pulsed flow micromixing

Goullet et al. examined the mixing of low Reynolds number laminar flows using pulsing and certain geometries. The effects of geometry on the mixing of the microfluids and the effects of pulsing on mixing properties were investigated. This work was conducted by means of computational fluid dynamics (CFD) using Fluent 6. The models contained 1.25mm of channel before a confluence and 3mm of channel after the confluence. This set up was investigated with different geometries in channels that were 200μm wide and 120μm deep. It is clear from this work that certain features in channels induce secondary flow and that superimposing pulsing on the flow from the inlets, out of phase from each other, leads to strong deformation of the interface between the two liquids. In addition to these conclusions, better mixing can be obtained by sending the fluids multiple times past the features that encourage mixing, see Figure 3.

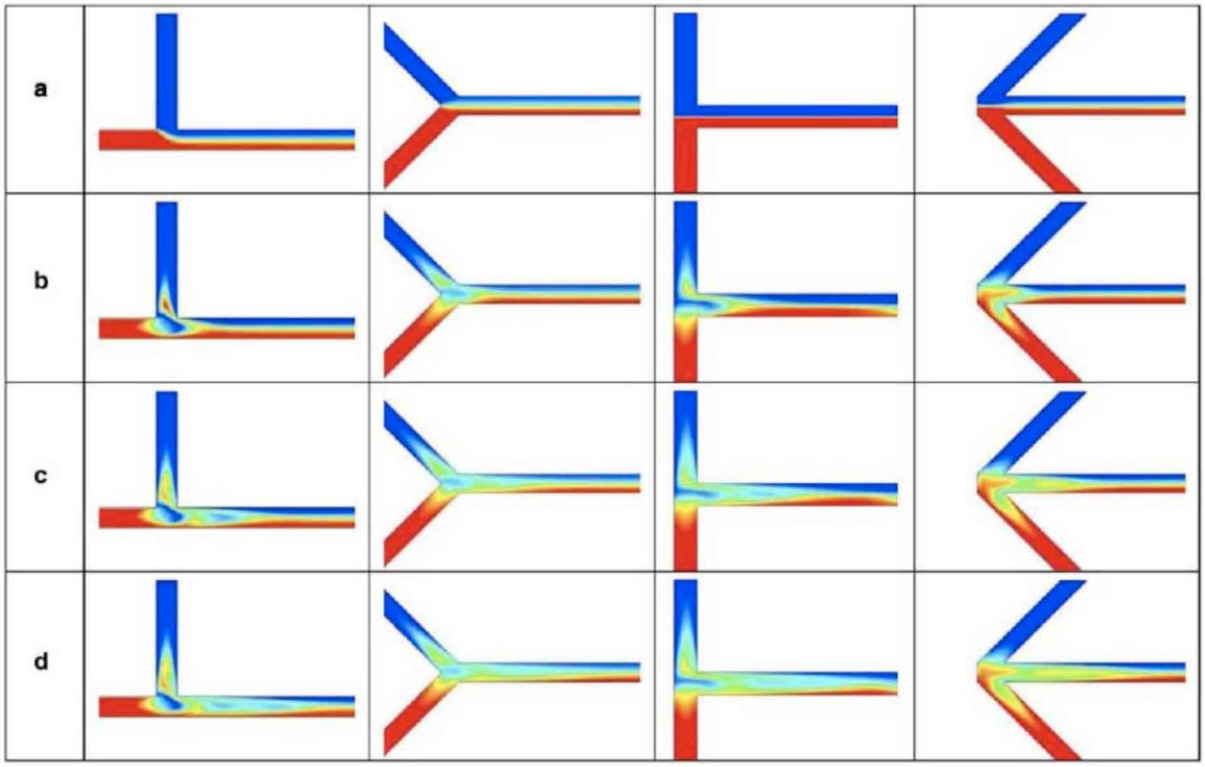

Figure 3: The data is for the case of 90° phase difference & 5 Hz pulsing at the same mean velocity of 1 mm×s^{-1} from each inlet after 1, 2 and 3 periods, respectively [18]

Interface configuration of the two layered laminar flow in a curved microchannel Yamaguchi et al. examined the fluid interface in curved microchannels [19]. The findings reinforce the theory of microchannels design, this design theory is based on three-dimensional comprehension of fluids and their associated mixing behaviour. In the experiments in this paper a microchannel was mechanically fabricated using a robotic drill with a flat end mill (diameter 200μm) used to cut 20mm straight channels, the straight channels were connected using curved channels of radii 500μm, as shown in Figure 4.

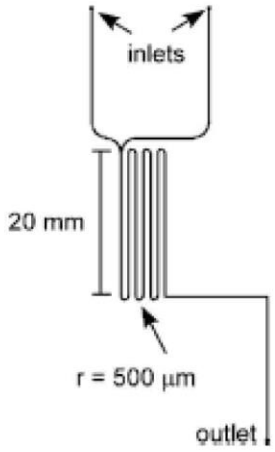

Figure 4: Top View of the Microchannel [19]

Purified water was run through one inlet and a fluorescein through the other. They merged to form a side by side parallel flow in the straight channel. Confocal fluorescence microscopy was then used to take pictures are different input velocities.

The CFD side of the investigation was conducted using Fluent 5 with the microchannel divided in 40 x 40 meshed cells. The circular arcs at the curves were divided in 120 mesh cells.

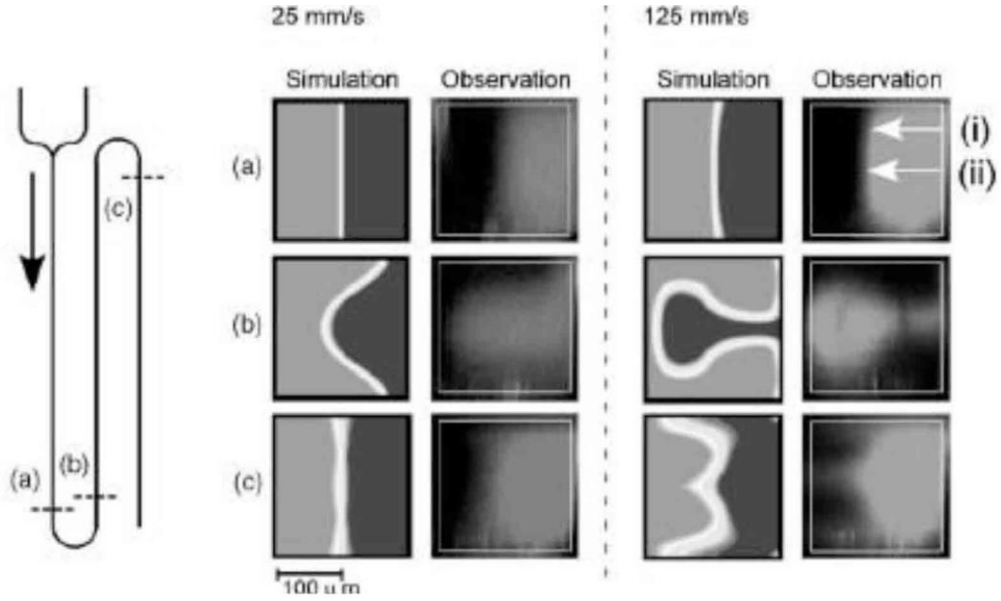

Figure 5: Comparison of simulation and observation experiment results for three-dimensional flow patterns [19]

The papers draw conclusions from the combined experimental and simulation work. The results reveal that the interface configuration of two liquids was affected by the secondary flow, induced

by inertial forces at the corners, see figure 5. It is shown that increasing velocity and density complicates the direction of particle movement in the flow, in contrast increasing curvature radius and viscosity simplified it.

Static mixing elements

Yamaguchi et al. investigated the effects of static mixing elements in a cross-shaped micromixer [19]. This geometry was examined to provide an answer to reaction kinetics problems. The complication with reaction kinetics studies is that when analyzing the reaction between enzymes and antibodies, large amounts of reagents are consumed. These enzymes are very expensive due to their limited availability. For these kinds of analysis to be viable the amount of reagent used must but be smaller than 1μl[20]. In their work a cross-shaped micro mixer was designed to utilize the mixing action of eddies and lateral velocities created by static mixing elements. The experimental configuration is shown is Figure 6 (a).

Using the CFD software CoventorWare^{TM} the model was drawn and solved. Verification was also carried out using Fluent 5.5^{TM}. In these CFD packages the geometry was drawn, then it was meshed using brick elements of 2μm, no-slip conditions were applied at the walls, and finally a pressure of 2 bars was applied to the inlets and a pressure of 2 x 10^{-4} bar was set at the outlet. The results showed that static mixing elements provided further improved mixing performance of the micromixer. The improvement was attributed to the eddy currents created as shown Figure 6 (b). The swirling of the eddy currents allows for larger contact areas between the fluids and reduced the diffusion distance to achieve uniform concentrations.

This modelling work showed that sub-millisecond mixing could be achieved with just 2 bar of applied pressure. This is a promising mixing technique for enhanced microfluids reaction kinetics [20].

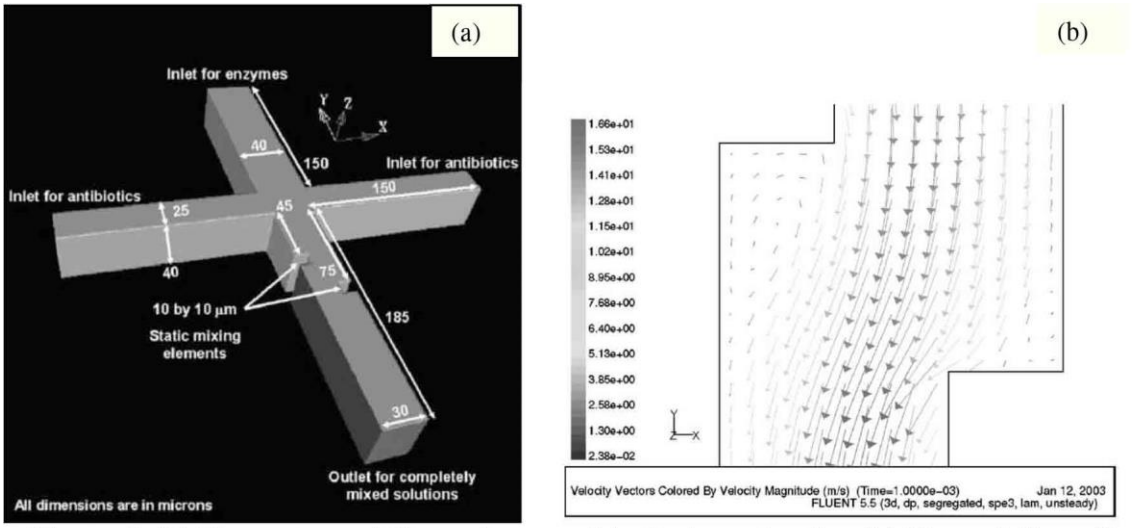

Figure 6: (a) Geometry and dimensions used in the investigation (b) The swirling effect and lateral velocity components that aid diffusive mixing [20]

Summary

It is clear from the previous applications discussed, that achieving uniform mixing is difficult for most microfluidic applications. There are many drives to make microfluidic mixing possible. For example, mixing on a small scale allows for smaller quantities of expensive fluids to be used. Applications like DNA separation and assay formation require quantities of expensive reagents. These enzymes and reagents are very expensive, so minimizing their use makes the processes much more commercially viable.

People are utilizing the eddy currents that are created from static mixing or curved geometries. When these currents are created, the mixing fluids are twisted and folded creating more surface to surface contact, allowing for more diffusive mixing and facilitating quicker mixing. This method allows, the use of shorter channels, keeps the volume usage and hence cost of expensive fluids low, and allows quicker results to be obtained. Optimum conditions would lead to better mixing and shorter settling times, and even possibly allows for a pressure reduction in these channels. Higher pressure is a method that has been used to increase Reynolds Number and increase mixing in many applications. Increasing pressures like this is a crude method for increasing mixing; and it can lead to systems becoming unstable and increases the need to more expensive valves and chips systems. From the application presented in the paper it can be seen that much work has been done on creating geometries to facilitate the mixing of fluids in these channels.

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